

***Distoseptispora longispora* sp. nov. from freshwater habitats in China**

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ABSTRACT—A novel species, *Distoseptispora longispora*, from submerged wood in Yunnan Province, China, is described and illustrated. The fungus is characterized by macronematous, mononematous, septate conidiophores with monoblastic, integrated, determinate, terminal conidiogenous cells that produce acrogenous, solitary, obclavate, elongated, distoseptate conidia. SSU-ITS-LSU sequence analyses were used to infer the phylogenetic relationship between *D. longispora* and related species. Both molecular analyses and morphological data well support *D. longispora* as an independent taxon. A key to *Distoseptispora* species is provided.

KEY WORDS—Ascomycota, asexual fungi, *Distoseptisporaceae*, hyphomycetes

Introduction

Distoseptispora K.D. Hyde & al. was introduced by Su & al. (2016) to accommodate two species, *D. fluminicola* McKenzie & al., the type species,

and *D. aquatica* Z.L. Luo & al. The genus is characterized by hairy colonies, macronematous conidiophores, monoblastic conidiogenous cells, and distoseptate acrogenous cylindrical conidia., *Distoseptispora* represents a phylogenetically distinct lineage in *Sordariomycetes* for which Su & al. (2016) proposed a new family, *Distoseptisporaceae* K.D. Hyde & McKenzie. Subsequently, 13 more species have been added: *D. cangshanensis* Z.L. Luo & al., *D. dehongensis* W. Dong & al., *D. guttulata* Jing Yang & K.D. Hyde, *D. martinii* (J.L. Crane & Dumont) J.W. Xia & X.G. Zhang, *D. obpyriformis* Z.L. Luo & H.Y. Su, *D. palmarum* S.N. Zhang & al., *D. phangngaensis* Jing Yang & al., *D. rostrata* Z.L. Luo & al., *D. submersa* Z.L. Luo & al., *D. suoluoensis* Jing Yang & al., *D. thailandica* Tibpromma & K.D. Hyde, *D. thysanolaenae* Goonas. & al., and *D. xishuangbannaensis* Tibpromma & K.D. Hyde (Hyde & al. 2019, Luo & al. 2018, Phookamsak & al. 2019, Tibpromma & al 2018, Xia & al. 2017, Yang & al. 2018).

Many aquatic fungi have been discovered in China’s freshwater habitats, which are rich in lignicolous fungi (Hu & al. 2007, 2012a,b; Huang & al. 2016; Song & al. 2018). Our ongoing survey of aquatic fungi in Yunnan Province revealed an interesting *Distoseptispora* species that we describe as new based on morphological characters and molecular analyses (SSU-ITS-LSU).

Materials & methods

Sample collection & morphological studies

Debarked wood samples from different freshwater habitats in Yunnan Province, China, were taken to the lab for detection of fungi using dissecting and compound microscopes. The dried specimens were deposited in the Herbarium of Fungi, Jiangxi Agricultural University, Nanchang, China (HFJAU).

DNA extraction, sequencing, and phylogenetic analyses

Genomic DNA was extracted from fresh fungal materials found on the natural substrate following Hu & al. (2012c). We amplified three rDNA regions: internal

TABLE 1. Isolates included in the phylogenetic analyses.

SPECIES	STRAIN	GENBANK ACCESSION NUMBERS		
		ITS	LSU	SSU
<i>Distoseptispora</i> “ <i>adscendens</i> ”	HKUCC 10820	—	DQ408561	—
<i>D. aquatica</i>	MFLUCC 15-0374 ^T	NR_154040	KU376268	—
	SNJ14	MH555360	—	—
<i>D. fluminicola</i>	MFLUCC 15-0417 ^T	NR_154041	KU376270	—
<i>D. guttulata</i>	MFLUCC 16-0183	MF077543	MF077554	MF077532

<i>D. "leonensis"</i>	HKUCC 10822	—	DQ408566	—
<i>D. longispora</i>	HFJAU 0705^T	MH555359	MH555431	MH555357
<i>D. martinii</i>	HSAUP myr4280	KU999975	KX033566	KX033537
<i>D. multiseptata</i>	MFLUCC 15-0609 ^T	NR_154017	KX710140	MF077540
	MFLUCC 16-1044	MF077544	MF077555	MF077533
<i>D. phangngaensis</i>	MFLUCC 16-0857	MF077545	MF077556	MF077534
<i>D. suoluensis</i>	MFLUCC 17-1305	MF077547	MF077558	MF077536
	MFLUCC 17-0224	MF077546	MF077557	MF077535
<i>D. tectonae</i>	MFLUCC 12-0291	KX751711	KX751713	—
	GZ25	MH555361	—	MH555358
<i>D. tectonigena</i>	MFLUCC 12-0292 ^T	NR_154018	KX751714	—
<i>Distoseptispora</i> sp.	HLXM 15-1	—	KU376269	—
<i>Dothidea sambuci</i>	AFTOL-ID 274	DQ491505	AY544681	AY544722
<i>Ellisembia bambusicola</i>	HKUCC 3578	—	DQ408562	—
<i>E. calyptrata</i>	HKUCC 10821	—	DQ408564	—
<i>E. minigelatinosa</i>	NN47497	—	DQ408567	—
<i>Ellisembia</i> sp.	HKUCC 10558	—	DQ408565	—
<i>Sporidesmium aquaticivaginatum</i>	MFLUCC 15-0624	KX710147	KX710142	MF077541
<i>S. aquaticum</i>	MFLUCC 15-0420	—	KU376273	—
<i>S. australiense</i>	HKUCC 10833	—	DQ408554	—
<i>S. fluminicola</i>	MFLUCC 15-0346	—	KU376271	—
<i>S. macrurum</i>	HKUCC 2740	—	DQ408555	—
<i>S. obclavatum</i>	HKUCC 10834	—	DQ408556	—
<i>S. olivaceoconidium</i>	MFLUCC 15-0380	KX710144	KX710139	MF077542
<i>S. pachyanthicola</i>	HKUCC 10835	—	DQ408557	—
<i>S. parvum</i>	HKUCC 10836	—	DQ408558	—
<i>S. pyriformatum</i>	MFLUCC 15-0620 ^T	KX710146	KX710141	—
	MFLUCC 15-0627	KX710148	—	KX710143
<i>S. submersum</i>	MFLUCC 15-0421	—	KU376272	—
<i>S. tengii</i>	HKUCC 10837	—	DQ408559	—
<i>S. thailandense</i>	MFLUCC 15-0964 ^T	MF374361	MF374370	—
<i>S. tropicale</i>	MFLUCC 16-0185	MF077551	MF077562	MF077539
	HKUCC 10838	—	DQ408560	—
<i>Sporidesmium</i> sp.	KUFA 0043	KT824799	—	—
<i>Sporidesmium</i> sp.	MFLUCC 15-0617	MF077550	MF077561	—
<i>Sporidesmium</i> sp.	MFLUCC 16-0186	MF077549	MF077560	MF077538

transcribed spacer (ITS) with the primer pair ITS1 & ITS4 (White & al. 1990), partial small subunit (SSU) with NS1 & NS4 (White & al. 1990), and large subunit (LSU) with LR0R & LR6 (Vilgalys & Hester 1990, Rehner & Samuels 1995). PCR protocols followed the conditions set by Hu & al. (2012b). The PCR products were purified and sequenced by the same primers used for PCR at Sangon Biotech (Shanghai) Co. Ltd.

In this study, we generated six novel sequences (MH555360, MH555359, MH555431, MH555357, MH555361 & MH555358) and retrieved 36 reference sequences from GenBank (TABLE 1) that were aligned using MAFFT v7.

The ML analyses were produced with RAXML v7.2.6 (Stamatakis & Alachiotis 2010) using a GTRGAMMA substitution model with 1000 bootstrap replicates and evaluated by bootstrap support (MLBS).

The best-fit evolutionary models were estimated using MrModeltest V2.2. Posterior probabilities (PP) (Zhaxybayeva & Gogarten 2002) were determined by Markov chain Monte Carlo sampling (BMCMC) in MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run 1,500,000 generations, with trees sampled every 100 generations and the first 25% deleted as burn-in. Posterior probabilities were calculated based on the remaining trees. The novel taxonomic descriptions and nomenclature were deposited in MycoBank (<http://www.mycobank.org/>).

Phylogenetic results

The six new sequences generated in our study included one LSU, two SSU, and three ITS sequences. The phylogenetic tree based on the three-locus analysis (FIG. 1) shows the relationship between the new taxon and other related species. The concatenated aligned dataset comprised 17 isolates from 13 *Distoseptispora* spp., 23 isolates from 21 *Sporidesmium* (or “*Ellisembia*”) spp., and one isolate of *Dothidea sambuci* (Pers.) Fr. (*Dothideomycetes*) as an outgroup. The dataset including alignment gaps comprised 2817 characters: 1348 for SSU, 629 for ITS, and 840 for LSU. The combined dataset ML tree (FIG. 1) with bootstrap support values (MLBS) and Bayesian posterior probabilities (BPP) indicates several well-supported clades, with the *Distoseptispora longispora* strain forming a well-supported clade (MLBS = 94%, BPP = 0.68) with other *Distoseptispora* species.

Taxonomy

Distoseptispora longispora H.Y. Song & D.M. Hu **sp. nov.**

FIG. 2

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Differs from *Distoseptispora aquatica* by its larger and more septate conidia; and from *D. tectonae* and *D. tectonigena* by its smooth and brown to yellowish brown conidia.

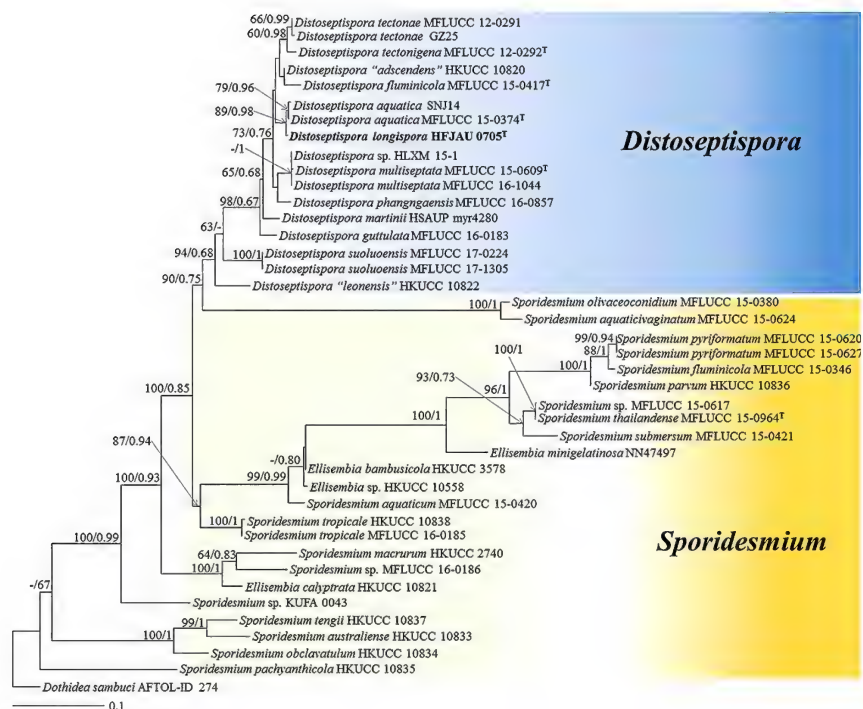


FIG. 1. Maximum Likelihood (ML) phylogenetic tree of *Distoseptispora longispora* and allied species based on concatenated SSU-ITS-LSU data. The RAxML bootstrap support values (MLBS) and Bayesian posterior probabilities (BPP) are given at the nodes (MLBS/BPP).

TYPE: China, Yunnan Province: Mengla, a small stream, on submerged wood, 13 Apr 2017, H.Y. Song (Holotype, HFJAU 0705).

ETYMOLOGY: *longispora*, referring to the long conidia of the fungus.

COLONIES on submerged wood superficial, effuse, scattered, hairy, brown to dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, subhyaline to pale brown hyphae. CONIDIOPHORES macronematous, mononematous, 2–4-septate, unbranched, single, straight or slightly flexuous, erect, cylindrical, smooth, brown to dark brown, $17\text{--}37 \times 6\text{--}10\ \mu\text{m}$. CONIDIOGENOUS CELLS monoblastic, integrated, determinate, terminal, light brown to dark brown, cylindrical. Conidial secession schizolytic. CONIDIA acrogenous, solitary, dry, obclavate, elongated, straight or slightly curved, rounded at the apex, truncate at the base, 31–56-distoseptate, smooth, brown

to yellowish brown, slightly paler towards the apex, $189\text{--}297 \times 16\text{--}23 \mu\text{m}$ (mean = $204 \times 21 \mu\text{m}$, $n = 50$).

ADDITIONAL SPECIMEN EXAMINED: CHINA, YUNNAN PROVINCE, Mengla, a small stream, on submerged wood, 13 Apr 2017, H.Y. Song (HFJAU 0706).

NOTES: *Distoseptispora longispora* is characterized by its macronematous, mononematous, septate conidiophores, monoblastic, integrated, determinate, terminal conidiogenous cells, and acrogenous, solitary, dry, obclavate, elongated, distoseptate conidia, which fit well with the generic concepts of *Distoseptispora* (Su & al. 2016). The molecular phylogeny (FIG. 1) clusters the *D. longispora* strain well within the *Distoseptispora* clade and closely related to *D. aquatica* but with an ITS six base pair (bp) difference separating it from *D. aquatica* (MFLUCC 15-0374). *Distoseptispora longispora* resembles *D. aquatica*, *D. tectonae*, and *D. tectonigena* in conidial shape. However, its larger and more septate conidia separate *D. longispora* from *D. aquatica* ($189\text{--}297 \times 16\text{--}23 \mu\text{m}$; 15–28 septa). In addition, *D. tectonae* has verruculose, dark reddish conidia, and *D. tectonigena* has dark reddish-brown conidia (Hyde & al. 2016).

The multilocus molecular phylogeny and morphological data fully support separation of our new species, *D. longispora*. Morphologically, *Distoseptispora* resembles *Ellisembia* Subram., first established to accommodate the species with distoseptate conidia in *Sporidesmium* Link (Subramanian 1992). However, a molecular phylogeny by Shenoy & al. (2006) showed that *Ellisembia* and *Sporidesmium* were polyphyletic. Su & al. (2016) later clarified differences between *Ellisembia* and *Sporidesmium*, noting that euseptate/distoseptate characters were not supported by molecular evidence and treating *Ellisembia* as a synonym of *Sporidesmium*. *Distoseptispora* species differ from *Ellisembia* (*Sporidesmium*) species in having relatively short conidiophores and darker conidia with slightly paler, but not hyaline, rounded apices, basal cells cut off by cross walls, and of indeterminate length (Su & al. 2016).

There are various hyphomycetes in freshwater habitats, and several studies have reported *Distoseptispora* species, e.g., *D. aquatica*, *D. fluminicola*, *D. guttulata*, *D. multiseptata*, *D. phangngaensis*, and *D. suoluensis* (Hu & al. 2007, 2010a,b; Huang & al. 2018; Hyde & al. 2016; Su & al. 2016; Yang & al. 2018). Most of these species were identified based on morphology, which proved to be insufficient for accurate identification (Hu & al. 2017). Numerous other studies (Réblová & al. 2016, Su & al. 2016, Zhang & al. 2017) have revealed many different freshwater lineages using molecular analyses.



FIG. 2. *Distoseptispora longispora* (holotype, HFJAU 0705). a. Colonies on submerged wood; b, c. Conidia; d. Conidiophore. Scale bars: a = 100 μm , b–d = 5 μm .

Key to *Distoseptispora* species

1. Conidia muriform, having both longitudinal and transverse septa *D. martinii*
1. Conidia with only transverse septa 2
2. Conidia euseptate 3
2. Conidia distoseptate 4
3. Conidia 75–130 × 7–11 µm, 11–14(–20)-euseptate, smooth *D. guttulata*
3. Conidia 80–125 × 8–13 µm, 8–10-euseptate, verrucose *D. suoluensis*
4. Conidiogenous cells polyblastic, conidia 35–180 × 7–11 µm *D. palmarum*
4. Conidiogenous cells monoblastic 5
5. Conidiophores having percurrently proliferating conidiogenous
cells at the apex of conidiophores 6
5. Conidiophores not as above 7
6. Conidia 115–155 × 9–11 µm *D. rostrata*
6. Conidia 148–225 × 11–12 µm *D. tectonigena*
7. Conidia verruculose, 7.5–9.5 × 3.5–5 µm *D. tectonae*
7. Conidia not verruculose 8
8. Conidia 3–5-distoseptate, 17–30 × 7.5–10 µm *D. dehongensis*
8. Conidia with more than 6 distosepta 9
9. Saprobiic on submerged wood in freshwater habitats 12
9. Not saprobiic on submerged wood in freshwater habitats 10
10. Saprobiic on dead culms of *Thysanolaena maxima*,
conidia 8–14-distoseptate, 21.5–80 × 6.5–12.8 µm *D. thysanolaenae*
10. Saprobiic on dead leaves of *Pandanus* sp. 11
11. Conidia reddish brown to brown, 130–230 × 13.5–17 µm *D. thailandica*
11. Conidia green-brown to brown, 160–305 × 8–15 µm *D. xishuangbannaensis*
12. Conidia 9–11-distoseptate, 53–71 × 12–16 µm *D. obpyriformis*
12. Conidia with more than 12 distosepta 13
13. Conidiogenous cells yellow, conidia 125–250 × 13–15 µm *D. fluminicola*
13. Conidiogenous cells brownish 14
14. Conidia brown to yellowish brown, 31–56-distoseptate,
189–297 × 16–23 µm *D. longispora*
14. Conidia greenish brown or dark olivaceous green 15
15. Conidiogenous cells subhyaline to pale brown,
conidia 58–166 × 10–14 µm *D. cangshanensis*
15. Conidiogenous cells brown to dark brown 16
16. Conidiogenous cells dark brown to olivaceous,
conidia 110–157 × 13.5–16.5 µm *D. aquatica*
16. Conidiogenous cells brown 17

17. Conidia dark olivaceous green, multi-distoseptate,
95–290 × 11–20 µm *D. multiseptata*
17. Conidia dark or mid olivaceous to mid or dark brown 18
18. Conidia dark olivaceous to mid or dark brown,
165–350 × 14–19 µm *D. phangngaensis*
18. Conidia mid-olivaceous to brown, 95–123 × 15–19 µm *D. submersa*

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